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EXPERIMENTAL STUDY OF ACUTE AND CHRONIC INJURY TO RENAL TUBULES

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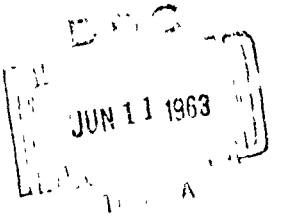

A B S T R A C T

1. Preparing Institution: Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Md.

2. Title of Report: Experimental Study of Acute and Chronic Injury to Renal Tubules.

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A study of the normal ultrastructure of the rat kidney has been undertaken as a basis for a study of the serial morphologic changes in acute tubular necrosis. By electron microscopy, a close structural relationship between endoplasmic reticulum and cytosomes and between cytosomes and the Golgi region. The cytosomes involved are acid-phospnatase containing and may be regarded, preliminarily, as lysosomes. The technique of nephron-dissection has been perfected in the laboratory and is being applied to normal kidney tissues.

AIM: Renal failure secondary to hemorrhage, shock, crushing injury, incompatible blood transfusion, burns, dehydration, etc. is well recognized clinically. The purpose of this study is to define the pathogenesis of renal damage in these states by determining the exact location of initial lesions, the specific mechanisms involved, and the relative significance of vascular, peripheral, tubular epithelial, and hemolytic components.

PROGRESS REPORT: (Work carried out by Dr. F. K. Mostofi of the Armed Forces Institute of Pathology and Dr. Robert H. Heptinstall of the Department of Pathology, Johns Hopkins).

A study of the normal ultrastructure of the proximal tubules of rat kidney was undertaken first as a basis for the contemplated study of the pathological changes in tubular necrosis induced by various experimental procedures. Emphasis was placed on the morphological characterization of different cytoplasmic inclusion bodies both by histochemistry and electron microscopy because of the role that they may play in cell necrosis.

Based on biochemical and histochemical observations some of the inclusion bodies are believed to contain hydrolytic enzymes. Biochemically, by differential ultra centrifugation, Strauss (J. Biophys, Biochem, Cytol. 2:745, 1956) isolated three different "droplet fractions" and demonstrated that they possessed high activities for cathepsin, acid phosphatase, B - glucoronidase and other acid hydrolases. Histochemically, as revealed by acid phosphatase reaction on the tissue sections, these enzymes seem to be located in droplets ranging in size from 0.5 - 3 microns in diameter. De Duve (Subcellular Particles, 128, 1959) has claimed that cellular necrosis is initiated as a result of the release of the hydrolytic enzymes from such inclusion bodies into the cytoplasm. This claim was based on the observation that there was an increase in unsedimentable lysosomal enzymes during the early stages of necrosis (as observed by biochemical methods). As acute renal failure is accompanied by visible necrosis of tubular epithelium it was deemed important to study the various types of inclusion bodies in normal tubules. In addition to confirmation of some of previous reports, certain original observations were made to obtain morphological characterization for the above biochemical and histochemical findings.

Normal young male Sprague-Dawley rats were anesthetized with nembutal intraperitoneally. In vivo fixation of the idneys was accomplished by dripping 1% OsO_4 solution onto the kidney surface for 20 minutes. Small pieces of the outer cortex then were fixed for another $1\frac{1}{2}$ hour in ice cold buffered 1% OsO_4, dehydrated and embedded in Epon. Ultrathin sections were either viewed unstained or stained with lead according to Karnovsky method in a Siemens Elmiskop I electron microscope, operated at 60 kv and with a 30 u or 20 u objective aperture.

With this type of fixation, the two subcapsular layers of tubules have open lumina and certain specialized areas of the cytoplasm are revealed. Such areas contain the Golgi apparatus, usually in association with single membrane-limited bodies of three different types:

A. Multivesicular bodies.

B. Large vesicular bodies, often containing dense material, ferritin-like granules and laminated membrane systems.

C. Bodies with homogeneous matrix and central dense "nucleoid" material.

The endoplasmic reticulum (ER) is relatively sparse. Two local specializations of the smooth ER occur:

1. Along lateral cell membranes a continuous tubular system extends from the apical region to the middle and basal parts of the cell.

2. Around the cytosomes of type C connections between these two types of specialized ER are also seen.

The findings indicate a close structural relationship between ER and cytosomes on the one hand and between cytosomes and the Golgi region on the other.

The specialized parts of ER associated with the lateral cell membranes may represent a transport system connecting apical cytoplasm with the intercellular space and with the Golgi-cytosome region. By comparing the size and distribution of the type B inclusion bodies and the acid phosphatase containing droplets (in histochemical sections) these inclusion bodies may be regarded as lysosomes.

Preliminary evidence appears to support this assumption and further studies are now being conducted to elucidate the nature of the lysosomes, which may play an important role in the development of cellular necrosis of several types.

Up to the present time the work performed by dissection of nephrons (Oliver) has been of a preparatory nature. Numerous preparations of normal kidneys have been made so that the various techniques involved, particularly dissection and photography can be perfected. Some diseased human kidneys particularly those with tubular lesions have been dissected and it has been shown that the "thyroid areas" seen so commonly in chronic pyelonephritis as part of the collecting system of the kidney and that many of these tubules are blind ended and sequestrated.

Preliminary papers have been presented in the following society meetings:

Walter Reed Monthly Nephrology Group.
Histochemical Society.
Anatomical Association.
International Academy of Pathology.

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